JOURNAL OF OPTOELECTRONICS AND ADVANCED MATERIALS Vol. 9, No. 3, March 2007, p. 756 - 759

The effect of pH on amino acids binding to gold nanoparticles

GH. MIHAILESCU[a*], L.OLENIC[a], S. PRUNEANU[a,b], I. BRATU[a], I. KACSO[a]
[a]*National Institute for Research and Development of Isotopic and Molecular Technologies, P.O. Box 700, 400293, Cluj-Napoca, Romania*
[b]*School of Science & Technology, University of Teesside, Middlesbrough Tees Valley TS1 3BA UK*

Biologically active molecules such as amino acids, peptides and proteins are usually attached to nanoparticles to improve their bio-specificity and to expand application potentialities of these types of systems in biological and medical sciences. The work reported here describes interactions between gold nanoparticles (GNP) and amino acids. The surface chemistry of particulate Au is dominated by its negative surface charge. The control of aggregation degree between amino acids and GNP is achieved by pH change of reaction environment. The aggregation process is followed by UV-Vis and IR spectroscopy. From the obtained data we calculated K- the association constants. The values of K observed in these experiments suggest a good association between GNP and amino acids. The paper presents the preliminary study in order to obtain 3D structures: GNP-amino acids with possible application as biosensors.

(Received November 15, 2006; accepted December 21, 2006)

Keywords: Gold nanoparticles, Amino acids, Multilayers

1. Introduction

Research on nanoparticle aggregates with organic compounds containing various functional groups has been attracting growing interest as a result of their collective electronic, optical, and magnetic properties [1] being distinctly different from a corresponding collection of individual nanoparticles [2] or the extended solid.

Self-assembled monolayers [3,4] (SAMs) provide a convenient system for adjusting the interfacial properties of metal, metal oxide or semiconductors. The ligands that form SAMs have a chemical functionality with a specific affinity for a substrate. A versatile procedure for the preparation of materials in a three dimensional form is the layer by layer (construction method). The "substrates" range from planar surfaces (glass, films of metal, single crystals) to highly curved nanostructures (colloids, nanocrystals, nanorods).

The criteria important for selecting the type of substrate and method of preparation are dependent on the application for which the nanoassembly is used.

Very often the gold is used as the best substrate for studying the characteristics of the new nanoassembly.

Gold nanoparticles (GNP) have excellent compatibility with biomolecules (amino acids, DNA) and the new architectures have a great potential to be used as biosensors.

Our intention is to develop a biosensor for amino acids. The work was focused on preparation and characterization of GNP (by an original method) and SAM-GNP-amino-acids, in order to obtain the aggregates as monolayer on different substrates (macro gold for electrochemical characterization and ITO for optical characterization) and 3D nanostructures, using layer by layer procedure.

2. Experimental

Chemicals: All aqueous solutions were prepared from bidistilled water. All amino acids were analytical grade and were obtained from Merck. Nanogold particles were obtained at INCDTIM-Cluj-Napoca after an original method.

A). GNP preparation and characterization

We have used an original method[5] to prepare GNPs by reducing a complex of Au^-, $Na_3Au(SO_3)_2$ with sodium citrate. The procedure provides control of the dimension of nanoparticles produced. In Fig. 1 one can see the TEM of GNPs used for construction of nanomaterials.

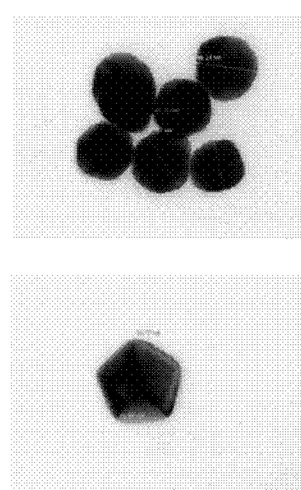

Fig. 1. Typical TEM image for GNPs.

We calculated the medium diameter of GNPs using the program « Scion Image » (www.scioncorp.com) The histogram is presented in Fig. 2 (the medium diameter is 31.3 nm).

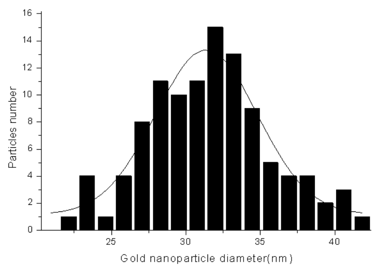

Fig. 2. The histogram of GNPs.

The concentration of GNPs solution was 0.6 nM.

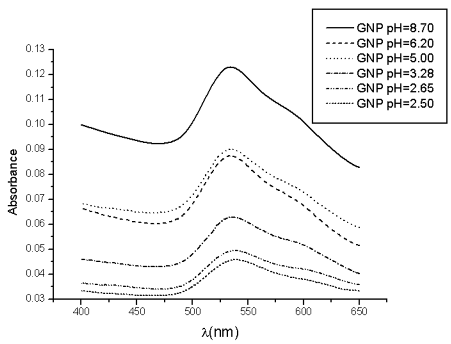

Fig. 3. UV-Vis spectra for GNP solutions at different pH (HCl: 0,01M).

B) GNP-amino acids aggregates preparation and characterization

Our intention is to develop two and three-dimensional arrays of gold nanoparticles capped with amino acids. The new materials will be used for electrochemical nanosensors.

The formation of gold aggregates from discrete particles capped with amino acids molecules depends on the pH of amino acid solution.

In this paper the effect of pH control is shown for some mono and bifunctional amino acids: glycine (Gly), alanine (Ala), cysteine (Cys), methionine (Met) and lysine (Lys).

3. Results and discussion

The aggregates between GNP and amino acid at pH: 2, 4, 6 and 8 were characterized by UV-Vis spectroscopy (Figs. 4,5)

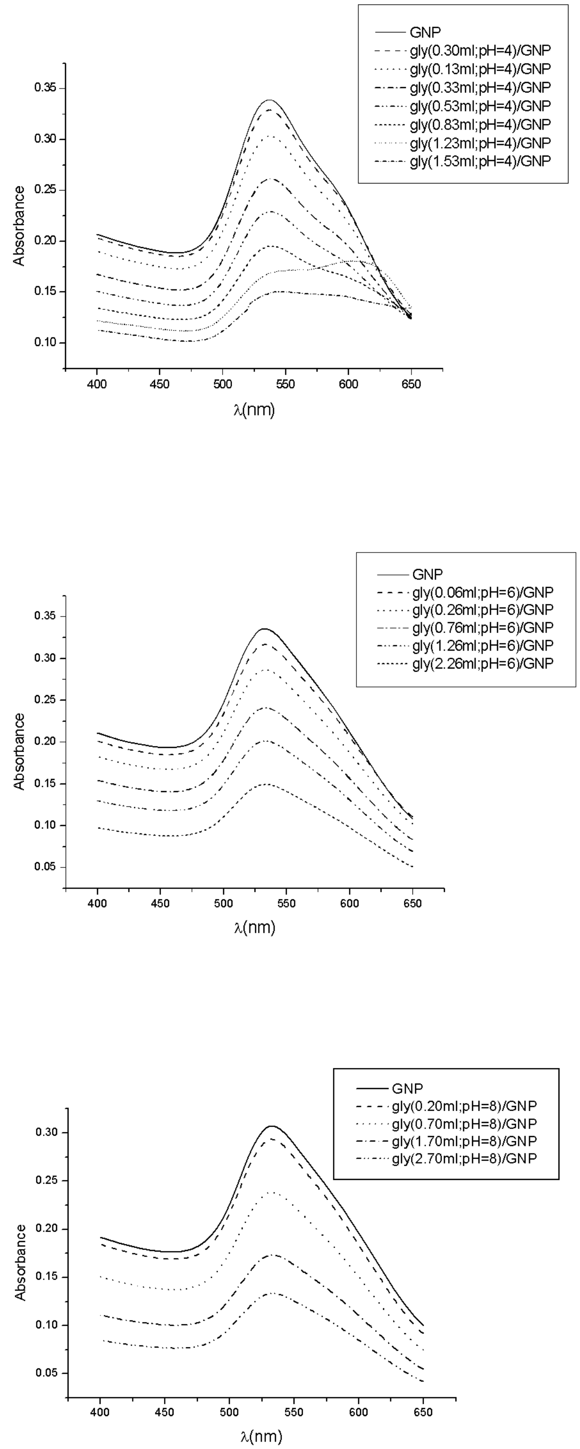

Fig. 4. UV-Vis spectra of Gly/GNP aggregates at: A-pH=4; B- pH=6; C- pH=8.

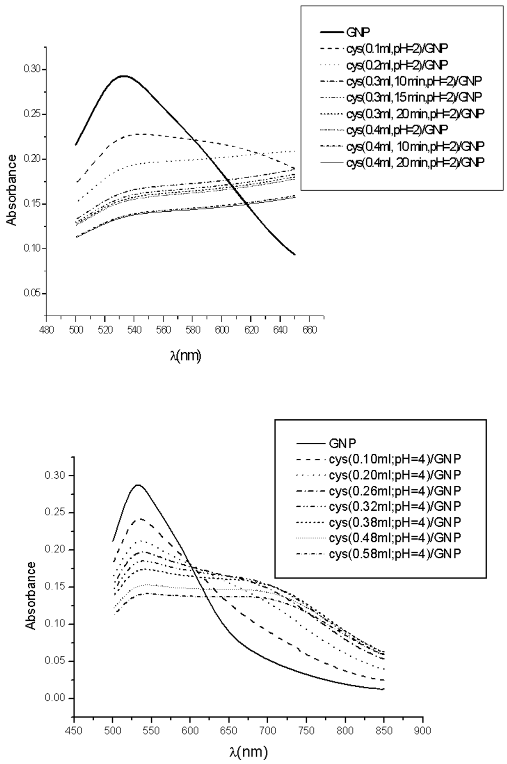

Fig. 5. UV-Vis spectra of Cys/GNP aggregates at: A- pH=2; B- pH=4.

It can be seen from UV-Vis spectra (Fig. 4, 5) that at pH=2 all amino acids form aggregates with GNP[6] (a clear and rapid bathochromic shift is observed that represent the diagnostic of aggregation); at pH=4 only Gly, Cys and Met and in neutral and basic medium they do not aggregate.

The isosbestic point at 600 nm confirm the existence of a complexation equilibrium.

The spectral changes observed in Fig. 6 can be used to assess the apparent association equilibrium under given set of medium conditions.

$$(Au^0)_n + AA \Leftrightarrow [(Au^0)_n \cdots AA]$$

(AA= amino acid)

From the equilibrium constant for the above reaction we obtained the equation:

$(Conc.GNP/absorbance)=[(1/K_{app})x(1/\varepsilon)x(1/conc.AA)+(1/\varepsilon)]$

K_{app} –complexation constant; ε –molar extinction coefficient

The calculated association constant[7] for Cys, at pH=4, is 1190 M^{-1}. The value of K_{app} calculated from the experiment suggests a good association between the GNP and cysteine.

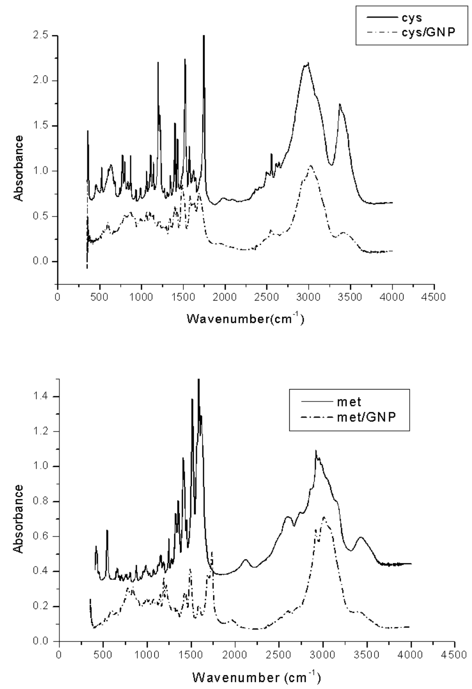

Fig. 6. IR spectra of : A- Cys and Cys/GNP aggregate; B-Met and Met/GNP.

IR spectra (Fig. 6) indicate the presence of the characteristic band of COO^- (≈1400 and 1600cm^{-1}), for amino acids (Cys and Met) and the presence of characteristic band C=O from COOH at 1720 cm^{-1} after amino acids aggregate with GNP.

The N-H stretching vibrations have very low intensity at 3400 cm^{-1} after aggregation of amino acids with GNP that confirm the association of GNP at this group. No peak could be seen at ≈2570 cm^{-1} (corresponding to the S-H stretching vibration mode) indicating that amino acids bind on the gold nanoparticles trough S-Au bonding.

4. Conclusions

The association between GNP and amino acids depends on the pH of amino acid solution.

In the solid state and in solutions amino acids are known to exist in the zwitterions form (NH_3^+-CH_2-COO^-).

At low pH the COO^- group is protonated at COOH and NH_2 at NH_3^+. These are the good conditions for gold nanoparticle (bearing surface negative charge) to bind the functional group (amine and thiol).

At high pH the amino acids exist as anions COO^- and NH_2 group remain unmodified. In this case the gold nanoparticle can not bind with the amino acid because of the negative charge of COO^- group.

The aggregates between GNP and amino acids at low pH make possible to design and investigate monolayers and 2D or 3D nanostructures as biosensors.

References

[1] W. U. Malik, R. N. Goyal, Indian J of Chemistry, **44A**, 861-874 (2005).
[2] R. Birringer, H. Gleiter, Encycl. of Mat. Sci. and Eng. 339, (1988).
[3] N. Nath, A. Chilkoti, Anal. Chem. **74**, 504-509 (2002).
[4] M. D. Musick, C. D. Keating, L. A. Lyon, S. L. Botsko, D. J. Pena, W. D. Holliway, T. M. McEvoy, J. N. Richardson, M. J. Natan, Chem. Mater. **12**, 2869-2881 (2000).
[5] L. Olenic, Gh. Mihailescu, S. Pruneanu, I. Bratu, G. Sigartau, L. B. Tudoran, Al. R. Biriş, D. Lupu, Particulate Science and Technology **23**, 1, 79 (2005).
[6] G. Mie, Annalen der Physik, 377, 1908.
[7] H. A. Benesi, J. H. Hildebrand, J. Am. Chem. Soc. **71**, 2703 (1949).

*Corresponding author: gigim@oc1.itim-cj.ro